6/130/06

In the United States Patent and Trademark Office

Appn. Number: 10/760,156
Parent Appn. 10/007,489 Filed: 12/05/2001
Applicant: Elizabeth Gay Frayne
Title: Use of a Modified Phosphate for Enhancing the Natural Mutation Rate in Bacteria and Mutating Recombinant DNA Phage Inserts.
Examiner: Nancy Vogel
Art Unit:1636

Assistant Commissioner for Patents
Washington, District of Columbia 20231

RE: Office Action Dated 4/21/06

Sir:

The patent considered is a divisional application of the parent application Ser. Nr. 10/007,489 filed 12/05/2001 titled *Microbial Production of Phosphorothioate Substituted DNA, RNA, and Oligo Mixtures*. The claims for this application correspond to claims 6-7 of the parent application number 10/007,489. I have modified them to meet the examiner's objection and have included an additional new claim. I have also modified the information disclosure statement to meet the examiner's objections.

Response to Claim Rejection 35 USC 112

I have shown in the parent application that thio-phosphate is readily incorporated into the nucleic acids of a variety of organisms. In the present application the basis for enhanced mutagenicity is the creation of phosphorothioate linkages in DNA. This can occur during the culture of bacteria, yeast, and fish in the presence of thio-phosphate. It is known from in vitro work (Kunkel et. al.) that such linkages impair the DNA editing ability of prokaryotic DNA polymerases. The editing ability results from exonuclease action in a 3'-5' direction to correct DNA mismatches. Phosphorothioate linkages in the DNA block the exonuclease activity and hence reduce the fidelity of DNA polymerase. Bacterial DNA

polymerases are fundamentally different from eukaryotic polymerases as the exonuclease or DNA editing activity is a part of the DNA polymerase itself. In general bacterial DNA polymerases are highly conserved. Therefore, it is reasonable to expect the method to similarly impair the DNA editing activity of DNA polymerases from other bacteria. In this regard, in vitro work has shown that phosphorothiate linkages impair the DNA editing activity of E. col DNA polymerase I as well as T4 DNA polymerase.

The results obtained with yeast can be explained in light of the fact that yeast like other eukaryotes contain a DNA editing activity that is separate from the polymerase. As such a different enzyme is involved in performing this function. This enzyme does not appear to be as sensitive to the presence of phosphorothioate linkages. A slight increase in the mutation rate was noted. Certainly the increase was not as dramatic as that in E. coli and as such is not likely to be as useful for enhancing mutagenesis rates.

Information Disclosure Statement

Page two has been modified according to the examiner's instructions. In addition a copy of the reference is provided.

Claims

Claim 6 and 7 have been amended. In doing so an additional claim 15 was added. Note the numbering is in reference to parent application and second divisional application.